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Author	Family Name	**Roest**
	Particle	
	Given Name	**Gemma**
	Suffix	
	Division	Laboratory for Molecular and Cellular Signaling, Department of Cellular and Molecular Medicine & Leuven Kanker Instituut
	Organization	KU Leuven
	Address	Leuven, Belgium
Author	Family Name	**Rovere**
	Particle	**~~La~~**
	Given Name	**Rita M.**
	Suffix	
	Division	Laboratory for Molecular and Cellular Signaling, Department of Cellular and Molecular Medicine & Leuven Kanker Instituut
	Organization	KU Leuven
	Address	Leuven, Belgium
Corresponding Author	Family Name	**Bultynck**
	Particle	
	Given Name	**Geert**
	Suffix	
	Division	Laboratory for Molecular and Cellular Signaling, Department of Cellular and Molecular Medicine & Leuven Kanker Instituut
	Organization	KU Leuven
	Address	Leuven, Belgium
	Email	geert.bultynck@med.kuleuven.be
Corresponding Author	Family Name	**Parys**
	Particle	
	Given Name	**Jan B.**
	Suffix	
	Division	Laboratory for Molecular and Cellular Signaling, Department of Cellular and Molecular Medicine & Leuven Kanker Instituut

Organization	KU Leuven
Address	Leuven, Belgium
Email	jan.parys@med.kuleuven.be

Abstract

The inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R) is a ubiquitously expressed Ca^{2+}-release channel localized in the endoplasmic reticulum (ER). The intracellular Ca^{2+} signals originating from the activation of the IP_3R regulate multiple cellular processes including the control of cell death versus cell survival via their action on apoptosis and autophagy. The exact role of the IP_3Rs in these two processes does not only depend on their activity, which is modulated by the cytosolic composition (Ca^{2+}, ATP, redox status, . . .) and by various types of regulatory proteins, including kinases and phosphatases as well as by a number of oncogenes and tumor suppressors, but also on their intracellular localization, especially at the ER-mitochondrial and ER-lysosomal interfaces. At these interfaces, Ca^{2+} microdomains are formed, in which the Ca^{2+} concentration is finely regulated by the different ER, mitochondrial and lysosomal Ca^{2+} transport systems and also depends on the functional and structural interactions existing between them. In this review, we therefore discuss the most recent insights in the role of Ca^{2+} signaling in general and of the IP_3R in particular in the control of basal mitochondrial bioenergetics, apoptosis, and autophagy at the level of inter-organellar contact sites.

Keywords (separated by '-')

Apoptosis - Autophagy - Ca^{2+} microdomains - Cell death - Cell survival - Endoplasmic reticulum - IP_3 receptor - Lysosomes - Membrane contact sites - Mitochondria

Chapter 7
IP_3 Receptor Properties and Function at Membrane Contact Sites

Gemma Roest, Rita M. La Rovere, Geert Bultynck, and Jan B. Parys

Abstract The inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R) is a ubiquitously expressed Ca^{2+}-release channel localized in the endoplasmic reticulum (ER). The intracellular Ca^{2+} signals originating from the activation of the IP_3R regulate multiple cellular processes including the control of cell death versus cell survival via their action on apoptosis and autophagy. The exact role of the IP_3Rs in these two processes does not only depend on their activity, which is modulated by the cytosolic composition (Ca^{2+}, ATP, redox status, ...) and by various types of regulatory proteins, including kinases and phosphatases as well as by a number of oncogenes and tumor suppressors, but also on their intracellular localization, especially at the ER-mitochondrial and ER-lysosomal interfaces. At these interfaces, Ca^{2+} microdomains are formed, in which the Ca^{2+} concentration is finely regulated by the different ER, mitochondrial and lysosomal Ca^{2+} transport systems and also depends on the functional and structural interactions existing between them. In this review, we therefore discuss the most recent insights in the role of Ca^{2+} signaling in general and of the IP_3R in particular in the control of basal mitochondrial bioenergetics, apoptosis, and autophagy at the level of inter-organellar contact sites.

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Gemma Roest and Rita M. La Rovere are Joint first authors.

G. Roest · R. M. La Rovere · G. Bultynck (✉) · J. B. Parys (✉)
Laboratory for Molecular and Cellular Signaling, Department of Cellular and Molecular Medicine & Leuven Kanker Instituut, KU Leuven, Leuven, Belgium
e-mail: geert.bultynck@med.kuleuven.be; jan.parys@med.kuleuven.be

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Abbreviations

ALS	Amyotrophic lateral sclerosis
AMPK	AMP-activated kinase
ATG	Autophagy-related
BIRD-2	Bcl-2/IP_3R disruptor-2 peptide
CICR	Ca^{2+}-induced Ca^{2+} release
CREB	cAMP response element-binding protein
DT40 TKO	DT40 IP_3R triple knock-out
ER	Endoplasmic reticulum
Fis1	Fission 1 homologue
GRP75	Glucose-regulated protein 75
GRP78/BiP	Glucose-regulated protein 78
GSK3β	Glycogen synthase kinase-3β
IMM	Inner mitochondrial membrane
IBC	IP_3-binding core
IP_3	Inositol 1,4,5-trisphosphate
IP_3R	IP_3 receptor
LC3	Microtubule-associated protein light chain 3
LRRK2	Leucine-rich repeat kinase 2
MAM	Mitochondria-associated ER membrane
MCU	Mitochondrial Ca^{2+} uniporter
Mfn	Mitofusin
mPTP	Mitochondrial permeabilization transition pore
mTORC1	Mechanistic target of rapamycin complex 1
OMM	Outer mitochondrial membrane
NAADP	Nicotinic acid adenine dinucleotide phosphate
PACS-2	Phosphofurin acidic cluster sorting protein 2
PERK	Protein kinase RNA-like ER kinase
PIP_3	Phosphatidylinositol 3,4,5-trisphosphate
PKB/Akt	Protein kinase B
PML	Promyelocytic leukemia
PTEN	Phosphatase and tensin homolog
PTPIP51	Protein tyrosine phosphatase-interacting protein-51
ROS	Reactive oxygen species
RyR	Ryanodine receptor
SERCA	Sarco-/endoplasmic reticulum Ca^{2+} ATPase
TCA	Tricarboxylic acid
TFEB	Transcription factor EB
TMX	Thioredoxin-like transmembrane protein
TPC	Two-pore channel
TRPML	Transient receptor potential mucolipin
ULK1/2	Atg1/Unc-51-like kinase 1/2
UPR	Unfolded protein response
VAPB	Vesicle-associated protein B
VDAC	Voltage-dependent anion channel

7.1 The IP_3 Receptor, the Main Ca^{2+}-Release Channel of the Endoplasmic Reticulum

Complex spatio-temporal Ca^{2+} signals regulate many fundamental cellular processes including fertilization, differentiation, proliferation, gene transcription, metabolism, contraction, secretion, etc. [1, 2]. The endoplasmic reticulum (ER) is the main Ca^{2+}-storage organelle and therefore plays a central role in intracellular Ca^{2+} signaling. ER Ca^{2+} handling depends on three major mechanisms, Ca^{2+} uptake via the sarco-/endoplasmic Ca^{2+}-ATPases (SERCA) [3], Ca^{2+} storage by various luminal Ca^{2+}-binding proteins [4] and controlled Ca^{2+} release. Besides passive Ca^{2+} leak through still largely unidentified basal ER Ca^{2+}-leak channels, Ca^{2+} release out of the ER primarily occurs via the inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R) and the ryanodine receptor (RyR). While the RyR is predominantly expressed in a limited number of tissues, especially muscles and brain [5], the IP_3R is ubiquitously expressed and present in virtually every cell type [6]. IP_3Rs are activated by IP_3 produced by phospholipase C after cell activation, e.g. by extracellular agonists, hormones, growth factors or neurotransmitters, and play a crucial role in the initiation and propagation of intracellular and intercellular Ca^{2+} signals [7–10].

In *Mammalia* and other higher organisms, three different genes encode IP_3Rs (*ITPR1*, *ITPR2* and *ITPR3*) giving rise to three isoforms named IP_3R1, IP_3R2, and IP_3R3 that display at the amino acid level an overall similarity of 75–80% [11]. Each of these isoforms is about 2700 amino acid long and assembles into tetrameric structures with a total molecular mass of 1.2 MDa. Splice isoforms and the possibility to form both homo- and heterotetramers increase the diversity between IP_3Rs. Each monomeric isoform has the same general structure containing five distinct functional domains (Fig. 7.1): the N-terminal coupling domain usually called the suppressor domain (for IP_3R1: a.a. 1–225), the IP_3-binding core (IBC, a.a. 226–578) containing the IP_3-binding site, the central coupling domain or modulatory and transducing domain (a.a. 579–2275), the channel domain containing six transmembrane helices (a.a. 2276–2589) and finally the C-terminal tail also named gatekeeper domain (a.a. 2590–2749) [13, 14].

At the structural level progress has followed two main paths. First, X-ray crystallography permitted to obtain high-resolution structural information of the N-terminal part of the IP_3R, i.e. the suppressor domain and the IBC [15, 16]. The latter contains two domains, a first one containing a β-trefoil fold (IBC-β) and a second one containing an armadillo repeat fold (IBC-α) with the cleft between them forming the IP_3-binding site. Their structure was also resolved at high resolution (3–3.8 Å) both for the apo- and the IP_3-bound form, elucidating changes occurring during IP_3R activation [17, 18]. In the presence of IP_3, a rearrangement of IBC-β and IBC-α occurs, constraining the IP_3-binding cleft. Additionally, the suppressor domain, originally in contact with both IBC-β and IBC-α, re-orients itself, probably to couple to the channel domain for its activation. Recently, Mikoshiba and co-workers succeeded in obtaining the crystal structure for the 2217 a.a. long cytosolic part of IP_3R1 at a resolution of 5.8–7.4 Å [19]. Comparison of the structures of

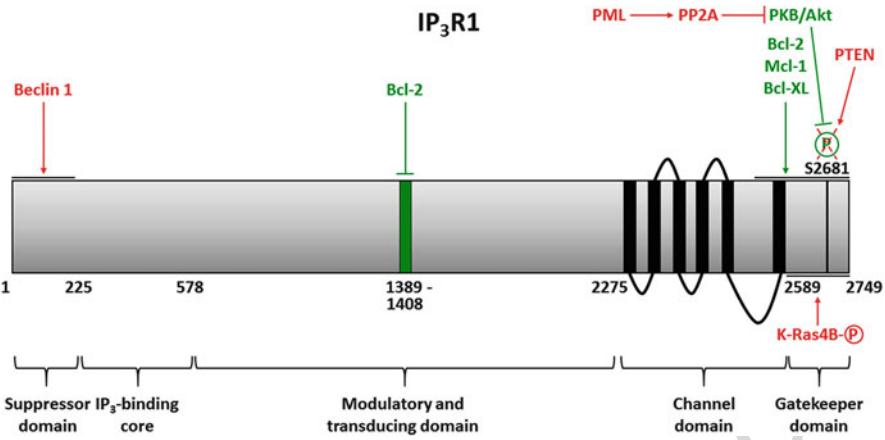

Fig. 7.1 Linear representation of the type 1 IP_3R isoform and its regulation by proteins involved in the control of cell survival and cell death. The five distinct IP_3R1 functional domains are delineated: the suppressor domain, the IP_3-binding core, the modulatory and transducing domain, the channel domain containing the six trans-membrane helices (indicated as black bars) and the gatekeeper domain. Oncogenes are represented in green, while tumor suppressors are indicated in red. Bar-headed lines indicate an inhibitory interaction and arrow-headed lines indicate a stimulatory interaction. For more information, see text. Reproduced with modifications from own previous work ([12], https://doi.org/10.1016/j.bbamcr.2016.01.002)

different truncated variants obtained in the absence and the presence of IP_3 supports a long-range gating mechanism in which the signal is transferred from the IBC via two α-helical domains (HD1, a.a. 605–1009 and HD3, a.a. 1593–2217) to a so-called leaflet (corresponding to a.a. 2195–2215 of HD3) that relays the IP_3R conformational change to the channel pore. Strikingly, the HD3/leaflet region was essential for the IP_3R Ca^{2+}-release activity in intact cells exposed to extracellular agonists.

Second, cryo-electron microscopy allowed gaining an increasingly better view of the full-length IP_3R structure. Serysheva and co-workers obtained by single-particle cryo-electron microscopy about 85% of the structure of rat cerebellar IP_3R1 at a 4.7 Å resolution in the apo-state [20, 21], confirming the close apposition of the N- and C-termini.

This structure enables the binding of four IP_3 molecules to a tetrameric IP_3R, but the exact stoichiometry underlying IP_3R opening by IP_3 remained for a long time elusive. The elegant work of Yule and co-workers, based on the ectopic expression in null-background HEK293 cells of concatenated IP_3Rs of which the IP_3-binding sites could be mutated, indicated that IP_3R-mediated Ca^{2+} release is only triggered when 4 IP_3 molecules are bound to the tetrameric IP_3R [22]. This stringent condition will not only prevent spurious IP_3R opening, but also enables the buffering of a significant amount of IP_3 molecules by the IP_3R channels themselves, ensuring that sufficiently high IP_3 concentrations are reached before IP_3R opening takes place [23].

Besides the physiological agonist IP_3, two metabolites produced by *Penicillium brevicompactum*, the adenophostins A and B, can activate the IP_3R at a much lower concentration than IP_3 itself [24]. Cytosolic Ca^{2+} is considered an important co-agonist of the IP_3R that acts in a bell-shaped dependent manner: a low [Ca^{2+}] (typically $\leq$0.3 μM) enhances IP_3-induced Ca^{2+} release, while a high [Ca^{2+}] (above 0.3 μM) suppresses IP_3-induced Ca^{2+} release [25–28]. ATP [29] and oxidizing conditions as e.g. thimerosal [30, 31] were also shown to stimulate IP_3-induced Ca^{2+} release. Finally, cAMP has been reported to sensitize IP_3Rs [32] independently of any phosphorylation event [33]. Moreover, very recently evidence was presented that when cAMP is produced, IP_3 can induce Ca^{2+} release from a different Ca^{2+} store that is not sensitive to IP_3 alone [34].

Interestingly, each of the IP_3R isoforms acts as a signaling hub, able to integrate various cellular inputs and to deliver a specific output signal that elicits a specific cellular response [9, 10, 35]. It should therefore be emphasized that although all IP_3R isoforms function along the same basic mechanisms, important differences in sensitivity exists between the various IP_3R isoforms, with IP_3R2 being the most atypical one [36]. The sensitivity of the IP_3R isoforms to IP_3 follows the rank-order $IP_3R2 > IP_3R1 > IP_3R3$ [37–41]. In addition, the sensitivity to cytosolic Ca^{2+} varies between the various IP_3R isoforms [42–44]. ATP similarly has a biphasic effect on IP_3R activity with between the IP_3R isoforms different sensitivities as well for the stimulatory [40, 45, 46] as for the inhibitory phase [47]. Moreover, IP_3R2, the isoform that is most sensitive to stimulatory ATP concentrations [45], proved to be dominant in a heterotetrameric receptor [48]. Finally, the oxidizing agent thimerosal has a biphasic action on IP_3R1 but only an inhibitory one on IP_3R3 [46, 49] while cAMP seems to have its main (or only) effect on IP_3R2 [32].

Inhibition of IP_3R activity is generally achieved by one of the following compounds: heparin, 2-APB, xestospongin C (or B) and caffeine. Except for the problem that all those compounds display non-specific effects [50], an additional problem is that they also may display a differential sensitivity between the various IP_3R isoforms [51]. The synthesis of IP_3 derivatives may however provide in the future more specific and/or more potent inhibitors [52].

Further regulation of IP_3R activity can be obtained by various post-translational modifications, including palmitoylation [53], cross-linking by the action of transglutaminase 2 [54] and phosphorylation/dephosphorylation events triggered by multiple protein kinases and protein phosphatases [55, 56]. Finally, the existence of multiple regulatory or scaffolding proteins able to associate with the IP_3R tetramer and to modulate its activity should be emphasized. Over 100 proteins have already been shown to interact with and regulate the IP_3R [57], including IRBIT [58, 59], calmodulin [60–64], neuronal CaBP1 [65–67], the anti-apoptotic Bcl-2 [68–70], Bcl-XL [71] and reticulocalbin 1 [72], the pro-autophagic Beclin 1 [73, 74], the chaperones ERp44 [75], GRP78/BiP [76] and sigma-1 receptor [77] and cytochrome c [78].

Many of these associated proteins, including several oncogenes and tumor suppressors (Fig. 7.1), are involved in the regulation of apoptosis and autophagy [12, 79]. This correlates with the important function of IP_3Rs and IP_3R-mediated Ca^{2+} signals in the control of cell-fate decisions, including processes related to cell death and survival

[80]. More specifically, the role of the IP_3R and of IP_3-induced Ca^{2+} release has been recognized in both apoptosis [81–86] and autophagy [85–90]. These processes depend on the activity of the IP_3R and its regulation by associated proteins and protein kinases, as well as on its intracellular localization, especially at the interface between ER and mitochondria or at the interface between ER and lysosomes.

7.2 Mitochondrial and Lysosomal Ca^{2+} Handling in Cell Death and Survival Processes

7.2.1 Apoptosis and Its Regulation by Ca^{2+}

Apoptosis is the major form of programmed cell death and can be initiated by either an extrinsic or an intrinsic –mitochondrial- pathway that both converge at the level of the activation of the effector caspases (caspase-3, -6 and -7) [91–93].

In the intrinsic pathway, Ca^{2+} overload of the mitochondrial matrix is a well-known factor leading to apoptosis [94]. Interestingly, several links exists between Ca^{2+} and apoptosis. In a first mechanism, mitochondrial Ca^{2+} is thought to bind cardiolipin, which thereby dissociates from mitochondrial complex II. The mitochondrial complex II disintegrates, leading to the release of the succinate dehydrogenase A and B subunits. This results in a massive reactive oxygen species (ROS) production and opening of the mitochondrial permeability transition pore (mPTP) [95]. This mPTP was proposed to arise from dimers of the F-ATP synthase in the inner mitochondrial membrane (IMM) [96, 97], though there still exists some controversy on the exact subunits involved [98]. Recent data indicate that mPTP opening can occur subsequently to Ca^{2+} binding to the β subunit of the catalytic F1 part [99]. This likely results in a conformational change transmitted via the oligomycin-sensitivity conferring protein subunit to the peripheral stalk and the IMM.

The mPTP is a high conductance channel that upon its opening results in the collapse of the electrochemical potential and in a loss of ion and solute distribution, terminating ATP synthesis. Subsequently to mPTP opening, solutes enter the mitochondrial matrix, leading to mitochondrial swelling, rupture of the outer mitochondrial membrane (OMM) and release of the pro-apoptotic factors located in the cristae, including cytochrome c. The release of these factors to the cytoplasm in turn leads to caspase activation and apoptosis [100].

Besides mPTP opening, Ca^{2+} can also indirectly activate mitochondrial apoptosis. Cytosolic Ca^{2+} can activate calcineurin, which dephosphorylates the sensitizer BH3-only protein Bad. This will cause its release from 14-3-3 proteins and allow its interaction with anti-apoptotic Bcl-XL. The latter will then be unable to counteract Bax/Bak activation and the subsequent permeabilization of the OMM will lead to apoptosis [101].

Finally, prolonged, severe depletion of the ER Ca^{2+} store will lead to ER stress and activation of the unfolded protein response (UPR) to re-establish ER

homeostasis. If the latter cannot be achieved, the UPR will eventually promote the expression of pro-apoptotic proteins, including members of the Bcl-2 family like BH3-only proteins, as well as that of GADD34 and of ERO1α, all leading to the cell demise by apoptosis [102, 103].

7.2.2 *Autophagy and Its Regulation by Ca^{2+}*

Autophagy is a highly conserved degradation pathway in which cellular components are targeted to the lysosomes. Basal and stress-induced autophagy act as survival pathways ensuring that protein aggregates, long-lived proteins, lipids, dysfunctional organelles or intracellular pathogens are digested and their constituents recycled, contributing to protein quality control, energy and cellular homeostasis [104]. Yet, excessive autophagy, brought about by the autophagy-inducing Tat-Beclin 1 peptide or by a severe/on-going nutrient starvation stress, can result in autophagic cell death (or so-called "autosis") via a process that is regulated by the Na^+/K^+-ATPase pump [105, 106].

Macroautophagy, generally referred to as autophagy, is the best-studied type of autophagy. It is characterized by the de novo formation of double-membrane vesicles named autophagosomes that engulf cytosolic proteins and/or organelles, the "cargo". These autophagosomes eventually fuse with the lysosome to form autolysosomes in which the cargo is degraded.

Main upstream controllers of autophagy are AMP-activated kinase (AMPK) and mechanistic target of rapamycin complex 1 (mTORC1). AMPK triggers autophagy in two ways, as it can phosphorylate and inhibit mTORC1, while directly activating the Atg1/Unc-51-like kinase 1/2 (ULK1/2) complex. The process downstream of ULK1/2 is further controlled by many proteins including about 30 autophagy-related (Atg) proteins [107, 108].

The initiation of the autophagy can start at various intracellular compartments, though the ER –and the ER-mitochondrial contact sites– seems to form a preferential location [109–111]. The original structure forming on the ER has been named the omegasome, which subsequently expands to form the phagophore [112]. Its subsequent elongation and closure, mediated by the Atg12-Atg5 complex, and the lipidation of microtubule-associated protein light chain 3 (LC3) by Atg4 and Atg8, lead to the formation of the autophagosomes. These ~~phagosomes~~ will eventually fuse either directly, or indirectly via prior fusion with an endosome, with lysosomes, forming hereby the autolysosomes needed for cargo degradation [107, 113].

The regulation of autophagy by Ca^{2+} appears quite complex as intracellular Ca^{2+} can stimulate or even be essential for autophagy [54, 73, 114–118] while it also can inhibit it [119–122]. Possible explanations to resolve the apparent controversy include the use of various cell types and various autophagy induction methods, and the fact that Ca^{2+} can act on multiple check points upstream of autophagy as well as during the autophagic process [85, 87–89, 123–127]. In that respect, the intracellular localization of the IP_3Rs appears important. Ca^{2+} signals directed to the

mitochondria will e.g. control mitochondrial metabolism and thus impact autophagy differently than Ca^{2+} signals directed to other targets. The importance for cell survival/cell death of IP_3R localization at membrane contact sites will therefore be discussed in the subsequent sections.

7.3 The Role of the IP_3R at ER: Mitochondrial Contact Sites

Ca^{2+} signals originating from the ER critically control cell death and survival by impacting the mitochondria [94, 128, 129]. This is possible through the existence of sites of close apposition between the ER and the mitochondria, covering about 10% of the mitochondrial surface, depending on the metabolic state of the cells [130]. These sites are based on the existence of structural connections between both organelles, resulting in the formation of mitochondria-associated ER membranes (MAMs). In those MAMs, numerous proteins were identified that play a role in Ca^{2+} handling, lipid transfer, inter-organellar tethering, regulation of mitochondrial fission and fusion, cell death control and mitochondrial metabolism [131–136].

The distance between both organelles is an important element in the control of cell death versus cell survival [134]. The contact sites are dynamic structures in which the distance between both membranes as well as the length of these contact sites vary in function of the physiological conditions. In general, the contact sites run over several hundreds of nanometers of length and the distance between smooth ER and mitochondria range between 10 and 50 nm and between rough ER and mitochondria between 50 and 80 nm [130]. By using synthetic linkers, ER-mitochondrial distance could experimentally be fourfold reduced increasing simultaneously fourfold the length of the ER-mitochondrial interface [137]. This resulted in an accelerated mitochondrial Ca^{2+} accumulation and an increased Ca^{2+}-dependent activation of the mPTP. Such tightening of the contact between ER and mitochondria physiologically occurs e.g. in cells undergoing apoptosis.

In physiological conditions, the distance between ER and mitochondria is determined by proteins responsible for tethering both organelles together. Although the molecular identity of the tethers remains less well understood in higher organisms than in yeast, several proteins contributing to ER-mitochondrial tethering have already been identified [133, 134, 138] (Fig. 7.2).

In the first place, one should mention that the ER-localized IP_3Rs are in the MAMs physically coupled to the voltage-dependent anion channels (VDAC) 1 located in the OMM via chaperones like glucose-regulated protein 75 (GRP75) [140] (Fig. 7.2a). This tethering enables an efficient "quasi-synaptic" Ca^{2+} transfer between ER and mitochondria [141]. This structure leads to high local [Ca^{2+}] (in the range of 10–20 μM) in the ER-mitochondrial interspace [142, 143], which overcomes the low-affinity properties of the mitochondrial Ca^{2+} uniporter (MCU) of the IMM [144]. Through the existence of these Ca^{2+} microdomains, the mitochondria

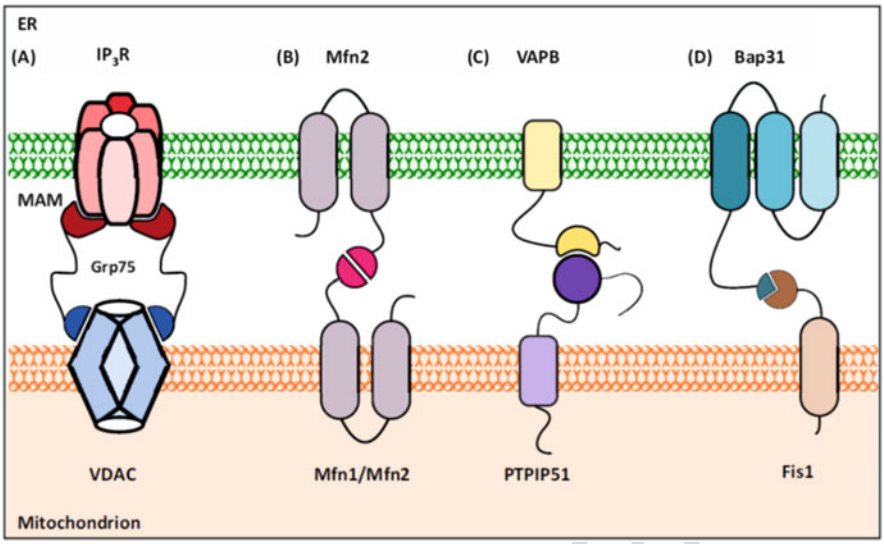

Fig. 7.2 The ER-mitochondrial tethers. Schematic visualization of the most important ER-mitochondrial tethers: (**a**) the IP_3R-GRP75-VDAC tether, (**b**) mitofusin-based tethering, (**c**) the VAPB-PTPIP51 tether and (**d**) the Bap31-Fis1 tether. For more information, see text. Reproduced from ([139], https://doi.org/10.1016/j.tins.2016.01.008) available through https://creativecommons.org/licenses/by/4.0/

can be regulated even by low-level Ca^{2+} signals. Dependently on the Ca^{2+} signal properties either cell survival or cell death can be promoted.

Mitofusin (Mfn) 2, enriched in the MAMs, was proposed to form homo- and heterodimers with mitochondria-localized Mfn2 and Mfn1 respectively (Fig. 7.2b), thereby establishing proper ER-mitochondrial apposition [145, 146]. Mfn2 function can be modulated by various proteins, including trichoplein/mitostatin, which counteracts Mfn2 function [147], and the ubiquitin ligase MITOL, which stimulates Mfn2 degradation [148]. In agreement with the model that Mfn2 has a tethering function, both trichoplein/mitostatin and MITOL impair mitochondrial Ca^{2+} uptake and protect against Ca^{2+}-dependent apoptosis. Other studies however have challenged the concept of Mfn2 as an ER-mitochondrial tether and actually proposed that it functions as an anti-tethering protein. This was based on the observation that cells lacking Mfn2 display an increased ER-mitochondrial tethering and an augmented agonist-induced mitochondrial Ca^{2+} uptake [149–152]. Resolving this controversy will probably depend on obtaining more detailed insights in the molecular composition of the ER-mitochondrial interface [153, 154].

Other proteins endowed with a role as tethers between the ER and the mitochondria are the scaffolds based on (a) vesicle-associated protein B (VAPB) in the ER interacting with protein tyrosine phosphatase-interacting protein-51 (PTPIP51) of the OMM, (b) on Bap31 in the ER and Fission 1 homologue (Fis1) in the OMM and (c) the ER stress sensor protein kinase RNA-like ER kinase (PERK) [133, 134, 138].

VABP is an integral ER membrane protein which mutation occurs in forms of motor neuron diseases including amyotrophic lateral sclerosis (ALS) [155]. Its interaction via its N-terminus with PTPIP51 (Fig. 7.2c) controls ER-mitochondria coupling and Ca^{2+} transfer between both organelles [156]. Moreover, the VAPB P56S mutant, which causes familial type 8 ALS [157], demonstrates both an increased binding to PTPIP51 and an increased Ca^{2+} flux to the mitochondria.

A pro-apoptotic interaction of Bap31 with Fis1 has been demonstrated (Fig. 7.2d), though the role of this interaction under normal physiological conditions is not yet understood [158]. The pro-apoptotic effect is due to the fact that this platform can both recruit pro-caspase-8 and increase Ca^{2+} transfer from the ER to the mitochondria. Moreover, the multifunctional phosphofurin acidic cluster sorting protein 2 (PACS-2) can modulate ER-mitochondria coupling and this has been related to Bap31 cleavage [159, 160].

Finally, PERK contributes to ER-mitochondria tethering independently of its canonical role in the UPR pathway, since in PERK-deficient cells the expression of a kinase-dead PERK was sufficient to re-establish ER-mitochondrial contacts and to re-sensitize them to apoptosis [161].

7.3.1 IP_3R-Mediated Ca^{2+} Signals in Cell Survival

Basal IP_3R-mediated Ca^{2+} signals usually in the form of Ca^{2+} oscillations- drive mitochondrial bioenergetics (Fig. 7.3a), especially by stimulating the activity of pyruvate dehydrogenase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase. These enzymes participate in the tricarboxylic acid (TCA) cycle and so control adequate ATP synthesis [162, 163]. In addition to this, mitochondrial Ca^{2+} can also enhance the activity of the ATP synthase complex V and of the adenine nucleotide translocator [164]. Furthermore, IP_3R-driven Ca^{2+} oscillations also sustain the mitochondrial metabolism by driving the transcription of the gene for MCU via regulation of the transcription factor CREB [165].

DT40 cells devoid of all IP_3R isoforms (DT40 TKO, ~~developed by~~ [166]) demonstrated in comparison to their wild-type counterparts a very different basic energy metabolism, with an increased Warburg effect and increased ROS production, explaining their reduced proliferation [167]. These effects may be aggravated by reduced MCU expression in cells lacking IP_3R expression and thus Ca^{2+} oscillations [165]. Inhibition of IP_3Rs in non-tumor cells led to a decreased ATP production, a subsequent activation of AMPK and therefore an increase in pro-survival basal autophagy [168]. The latter effect might depend on growth conditions as the increase in AMPK activity in DT40 TKO cells was observed in one study [168] but not in another [167]. Interestingly, similar results were obtained when ER-mitochondria coupling was impaired by downregulation of VABP or of PTPIP51 [169]. Tightening of the ER-mitochondria connection by a synthetic linker or by overexpression of either VABP or PTPIP51 reversed the effect. However, overexpression of the tether proteins only impaired autophagy induced by mTORC1

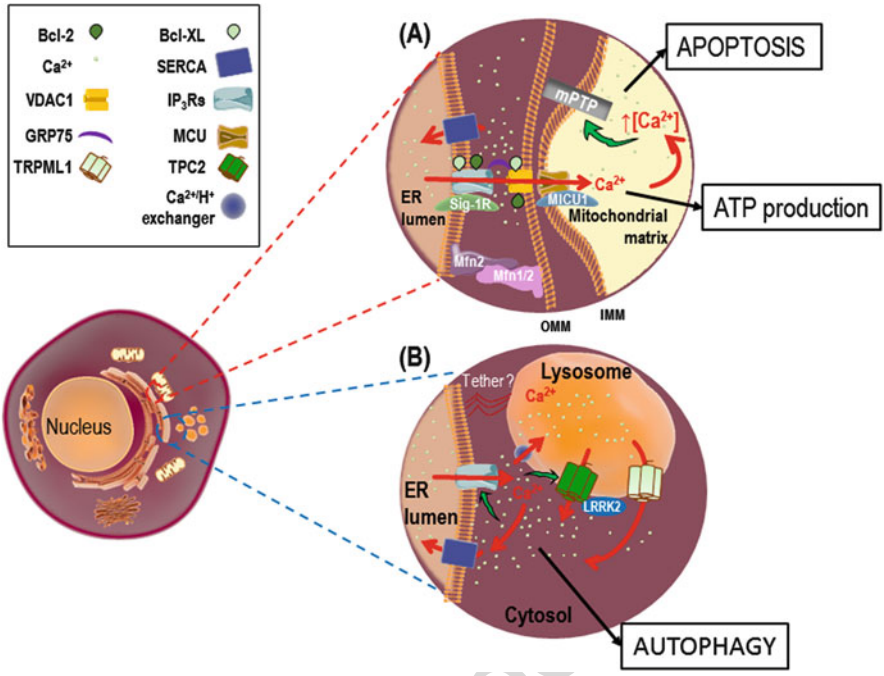

Fig. 7.3 Ca^{2+} transfer between ER and mitochondria and between ER and lysosomes. Schematic representation of Ca^{2+} handling in juxta-ER microdomains. (**a**) Ca^{2+} transfer to the mitochondria is mediated by the close connection between the IP_3R and in the ER and VDAC in the outer mitochondrial membrane (OMM). Moreover, additional tethers (the mitofusin tether is depicted) contribute to the close apposition of ER and mitochondria. The Ca^{2+} in the mitochondrial matrix drives mitochondrial bio-energetics via stimulation of the TCA cycle, the ATP synthase complex V and the adenine nucleotide translocator. In case of mitochondrial Ca^{2+} overload, the mPTP is opened, causing mitochondrial swelling and apoptosis. (**b**) Regulation of the Ca^{2+} concentration in the microdomain between ER and lysosomes involves both the IP_3R in the ER membrane and the lysosomal Ca^{2+} channels as TRPML1 and TPC2. Lysosomal Ca^{2+} uptake likely occurs via a Ca^{2+}/H^+ exchanger and molecular identification of ER-lysosomal tethering is presently lacking. The Ca^{2+} in the microdomain between ER and lysosomes can regulate the autophagic process upstream of autophagosome formation, during autophagic flux and via calcineurin-mediated activation of transcription factor EB. Ca^{2+} fluxes are depicted by red arrows. The green arrows indicate stimulatory interactions. For more information, see text.

inhibition and not nutrient starvation-induced autophagy, uncovering differences between the role of mitochondrial Ca^{2+} handling in different types of autophagy.

In contrast, the survival of tumorigenic cells depends on the IP_3Rs as adequate Ca^{2+} signaling is necessary to sustain mitochondrial metabolism [170, 171]. Although tumor cells proliferate in an uncontrolled way independently of the mitochondrial bioenergetics, they need the TCA cycle for the production of metabolites as nucleotides for cell growth and proliferation. The AMPK activation and the subsequent autophagy occurring after IP_3R inhibition is therefore not sufficient to sustain cell survival, resulting in a mitotic catastrophe.

Of note, the net effect of IP_3Rs on mitochondrial bio-energetics will depend on the local activity of the SERCA pumps at the MAMs (Fig. 7.3a), which, by pumping Ca^{2+} back into the ER, will limit the available Ca^{2+} in this microdomain and thus counteract ER-mitochondrial Ca^{2+} transfer. The local SERCA activity can be dynamically regulated, for instance by associated proteins like thioredoxin-like transmembrane protein (TMX) 1 [172, 173]. This redox-sensitive oxidoreductase is enriched at the MAMs, locally inhibiting SERCA pump activity and thus causing a local increase in $[Ca^{2+}]$ at the ER-mitochondrial interface and enhancing oxidative phosphorylation. In contrast, low levels of TMX1 will enhance SERCA activity, shifting Ca^{2+} away from the mitochondria and from the Ca^{2+}-driven bio-energetic pathway. In fact, loss of TMX1 has been observed in tumor cells, favoring their growth by potentially contributing to the Warburg effect. Nevertheless, a low-level ER-mitochondrial Ca^{2+} transfer must remain present to sustain proper TCA cycling and the production of the mitochondrial intermediates necessary for anabolic processes that ensure cell survival upon cell division [80, 170].

Apart from a regulation of autophagy, mitochondrial Ca^{2+} also impacts mitophagy, the specific elimination of mitochondria via the autophagic process. In fibroblasts expressing a mutation in the electron transport chain leading to a mild phenotype, mitochondrial Ca^{2+} uptake decreased, leading to increased autophagy, increased mitophagy and increased biogenesis of new mitochondria [174]. This adaptive response may account for the limited phenotype observed in these cells. In human RPE1 cells, ER-mitochondrial contacts appeared necessary for starvation-induced autophagy and for mitophagy [175]. The sigma-1 receptor located at the MAMs was crucial for starvation-induced autophagy. Its downregulation, however, did not affect mitophagy, and neither did the downregulation of all the RyR isoforms, of all the SERCA isoforms, or of any of the three IP_3R isoforms. Since the combined knockdown of all three IP_3R isoforms or of the MCU inhibited mitophagy, this specifically indicates the importance of IP_3R-mediated Ca^{2+} transfer from the ER to the mitochondria. Noteworthy, the sigma-1 receptor at the MAMs stabilizes IP_3R3 and so regulates Ca^{2+} transfer between ER and mitochondria [176] (Fig. 7.3a).

The anti-apoptotic proteins Bcl-2, Bcl-XL and Mcl-1, of which at least the first two have been identified in the MAM fraction [136] (Fig. 7.3a), can bind to the gatekeeper domain in the C-terminal region of the IP_3R (Fig. 7.1), which is activated by its interaction with the N-terminal IP_3-binding domain [177]. Their binding leads to a sensitization of the IP_3R, increased transfer of Ca^{2+} to the mitochondria and therefore an increased production of ATP, promoting cell survival [69, 71, 178]. Recent work uncovered the existence of a biphasic action of Bcl-XL on the IP_3R, i.e. a high-affinity activation at low Bcl-XL concentration and a lower affinity inhibition at high Bcl-XL concentrations [179]. Finally, it should be mentioned that Bcl-XL bound to the IP_3R C-terminus in the presence of active K-Ras4B phosphorylated by protein kinase C, a trimolecular complex ~~is formed~~ that antagonizes the anti-apoptotic action of Bcl-XL and promotes cell death via excessive autophagy [180].

7.3.2 IP_3R-Mediated Ca^{2+} Signals in Apoptosis

In contrast, excessive IP_3R-mediated Ca^{2+} release directed to the mitochondria will lead to mitochondrial Ca^{2+} overload and eventual apoptosis, as discussed above (Sect. 7.2.1). Especially IP_3R3 [181] and VDAC1 [182] were proposed to play an important role in pro-apoptotic Ca^{2+} signaling, but depending on the cell type, also other IP_3R isoforms can participate in such pro-apoptotic Ca^{2+} signaling [183, 184]. Moreover, besides IP_3Rs, other Ca^{2+}-transport systems that can impact ER-to-mitochondria Ca^{2+} flux can be enriched in the MAMs, including SERCA pumps [185] and S1T, a truncated form of SERCA1, induced upon ER stress and unable to pump Ca^{2+} [186]. By forming an ER Ca^{2+}-leak pathway, S1T expression leads to ER depletion, mitochondrial immobilization and increased ER-mitochondrial contact sites leading to increased mitochondrial Ca^{2+} levels and apoptosis.

At the level of the MAMs, multiple proteins, including oncogenes and tumor suppressors, regulate IP_3R activity and thus ER-mitochondria Ca^{2+} transfer and impact the apoptosis process [12].

Various anti-apoptotic Bcl-2 family members (Fig. 7.3a), including Bcl-2, Bcl-XL and Mcl-1, can modulate IP_3R activity [12, 187–191]. Bcl-2 binds to all three IP_3R isoforms at a conserved stretch of 20 amino acids located in the middle of their modulatory and transducing domain (Fig. 7.1), inhibiting IP_3R-mediated Ca^{2+} release [192]. Bcl-2 in this way prevents pro-apoptotic Ca^{2+} signaling. The BH4 domain of Bcl-2 is hereby sufficient to inhibit IP_3Rs and to protect cells against Ca^{2+}-dependent apoptosis [70]. Of note, the BH4 domain of Bcl-2 appears to be unique compared to the related domain present in Bcl-XL, which was much less effective in binding to and inhibiting IP_3Rs [193]. In contrast, the BH4 domain of Bcl-XL appeared more effective for suppressing VDAC1-mediated pro-apoptotic Ca^{2+} transfer to the mitochondria [194].

Consequently, disrupting ~~this~~ interaction by using a peptide corresponding to the binding site of Bcl-2 on IP_3R1 resulted in increased IP_3R activity and apoptosis [192]. Based on this binding site, the cell-permeable Bcl-2/IP_3R disruptor-2 peptide (BIRD-2) was developed [195]. It elicited spontaneous pro-apoptotic Ca^{2+}-release events in various types of cancer cells, including chronic lymphocytic leukemia [195], diffuse large B-cell lymphoma [183], multiple myeloma and follicular lymphoma [196] and small cell lung cancer [197].

IP_3R activity can also be reduced by phosphorylation by the pro-survival protein kinase B (PKB/Akt) thereby preventing pro-apoptotic Ca^{2+} release [198–200]. The PKB/Akt phosphorylation site is conserved among all three IP_3R isoforms (Fig. 7.1), but a more selective role for PKB/Akt in the regulation of IP_3R3 was proposed, as a study demonstrated that PKB/Akt could only dampen the Ca^{2+} signals in cells expressing that isoform [201]. The phosphatase and tensin homolog (PTEN), a phosphatidylinositol 3,4,5-trisphosphate (PIP_3) phosphatase upstream of PKB/Akt, counteracts the effect of PKB/Akt on IP_3Rs while re-expression of PTEN in PTEN-deficient cancer cells restored their sensitivity to pro-apoptotic Ca^{2+}-dependent stimuli [200]. Further studies indicated a direct role for PTEN on

IP_3R phosphorylation and function at the MAMs, where PTEN functioned as a protein phosphatase counteracting PKB/Akt-mediated phosphorylation of IP_3R3 channels and augmenting ER-mitochondrial Ca^{2+} flux [202]. In addition to this, it was very recently discovered that PTEN promotes Ca^{2+}-induced apoptosis in a phosphatase-independent way [203]. Indeed, PTEN competes with the F-box protein FBXL2, an ubiquitin E3 ligase component belonging to the SCF (SKP1, Cullin 1, F-box protein) E3 ligase family [204] with known tumor suppressor action by arresting the cell cycle [205, 206], for binding to the IP_3R3 channels. FBXL2 is responsible for IP_3R3 ubiquitination and its subsequent proteasomal degradation. As a consequence, PTEN, by competing with FBXL2, stabilizes IP_3R3 and promotes continuous Ca^{2+} transfer to the mitochondria leading to apoptosis. Finally, the negative regulation of IP_3R3 by PKB/Akt is antagonized by the tumor suppressor promyelocytic leukemia (PML) that recruits protein phosphatase PP2A to the MAMs. The latter dephosphorylates PKB/Akt, inhibiting its kinase activity and thus suppressing PKB/Akt-mediated phosphorylation of IP_3Rs (Fig. 7.1). Through this mechanism, PML increases Ca^{2+} release by the IP_3R, supporting mitochondrial Ca^{2+} overload and apoptosis [207].

In the heart, it was demonstrated that cyclophilin D participates to the IP_3R-GRP75-VDAC complex and regulates Ca^{2+} transfer to the mitochondria [208]. Its genetic or pharmacological inhibition attenuated mitochondrial Ca^{2+} overload and protected the cells from the consequences of hypoxia-reoxygenation.

Glycogen synthase kinase-3β (GSK3β) is another regulator of the IP_3R located in the MAMs [209]. GSK3β phosphorylates the IP_3R and increases IP_3-induced Ca^{2+} release. Upon ischemia-reperfusion injury, GSK3β activity is increased in cardiomyocytes, leading to increased pro-apoptotic Ca^{2+} signaling towards the mitochondria and cell death. Conversely, pharmacological inhibition of GSK3β protects the heart against damage subsequent to ischemia-reperfusion injury.

Finally, it should also be mentioned that increased transfer of Ca^{2+} to the mitochondria can also lead to cellular senescence. Adequate Ca^{2+} transfer to the mitochondria via IP_3R2 and the MCU appeared important in oncogene-induced senescence and replicative senescence [210].

7.4 The Role of the IP_3R at ER: Lysosomal Contact Sites

Lysosomes do not only play a fundamental role in the degradation of proteins and organelles, but are also bona fide Ca^{2+} stores [211–213] containing Ca^{2+} at a concentration of about 500 μM while their pH is between 4 and 5 [214]. Ca^{2+} uptake in the lysosomes is usually assumed to be mediated by a Ca^{2+}/H^+ exchanger [215] though other possibilities exist. In contrast to the uncertainty concerning the uptake mechanism, multiple Ca^{2+}-release channels are known to be present in the lysosomes, including transient receptor potential mucolipin (TRPML) 1 and two-pore channel (TPC) 2 [216]. Moreover, the lysosomes were identified as a target for nicotinic acid adenine dinucleotide phosphate (NAADP), a very potent agent eliciting Ca^{2+} release even at nanomolar concentrations (for a recent review, please

see [217]). With respect to the Ca^{2+}-releasing agent NAADP, most evidence point towards channels of the TPC family as their target but other channels, including TRPML1, have also been proposed. Further work is needed to fully understand the function of the various TPC channels, as work by various groups have shown that they can permeate several ions, including Ca^{2+}, Na^+ and H^+, and that their properties can be strongly dependent on experimental conditions and/or the presence of regulatory factors [218, 219].

As the autophagy process is regulated by Ca^{2+} (see above, Sect. 7.2.2) and as the Ca^{2+} ion has only a short range of messenger action [220], it can be assumed that the Ca^{2+} concentration in the microdomain between the ER and the lysosomes plays an important role in the control and/or fine-tuning of the autophagic process. This concentration will depend on the relative activity of the Ca^{2+} transporters in ER and lysosomes, their mutual functional interaction and the relative distance between the two organelles [85] (Fig. 7.3b).

In that respect, it is important to note that the two main Ca^{2+} channels expressed in the lysosomes, i.e. TRPML1 [221–223] and TPC2 [219, 224, 225] have already been implicated in autophagy (see further below).

As is the case for the mitochondria, also a very close association between ER and lysosomes was documented. Extensive contact sites between the two organelles were visualized through electron microscopy, showing in primary human fibroblasts that over 80% of the lysosomes are forming contacts of <20 nm with the ER [226]. This close connection should allow for mutual functional interactions between ER and lysosomes. In rat pulmonary artery smooth muscle cells, the close contacts between lysosomes and the sarcoplasmic reticulum allow for the functional coupling of lysosomes to RyR3 via Ca^{2+}-induced Ca^{2+} release (CICR) [227]. The contact sites involved show a membrane separation of about 15 nm apart, and extend for over 300 nm; increasing the membrane separation to 50 nm leads to a failure in activating RyR3. In contrast to what is known about other contact sites, the molecular mechanisms responsible for ER-lysosomal tethering are still only very partially recognized [228, 229].

Several studies have demonstrated that a small amount of Ca^{2+} released by NAADP can trigger much larger Ca^{2+} signals by activation of IP_3Rs or RyRs located on the ER by CICR or by ER Ca^{2+} store overload [226, 230–232] (Fig. 7.3b). The converse situation is also possible and Ca^{2+} released by the ER can affect lysosomal Ca^{2+} handling by stimulation of either NAADP synthesis or of the NAADP-induced Ca^{2+} release [233]. The Ca^{2+} ions released by the IP_3R can also be taken up in the lysosomes, leading to a dampening of the Ca^{2+} signals [234, 235]. A similar functional interaction also exist between active TRPML1 channel and ER Ca^{2+} release [236].

Taken together, this indicates that Ca^{2+} microdomains exist between the ER and the lysosomes in which the local Ca^{2+} concentration is regulated in a very complex way, whereby the IP_3R plays an important role [85, 213, 237–240] (Fig. 7.3b). These Ca^{2+} microdomains can therefore regulate the autophagy process. Evidence for stimulatory [241, 242] and inhibitory [243, 244] effects of lysosomal Ca^{2+} release on autophagy were presented. These differences in effects can be due to the use of different cellular models and of different mechanisms of autophagy induction differentially affecting the

relation between ER and lysosomes, but can also be an indication that Ca^{2+} can impact in a different way the various stages of the autophagic pathway. Moreover, the various lysosomal Ca^{2+} channels have been linked to different parts of the autophagy process. The absence of TRPML1 led to an upregulation of autophagy induction but an impaired lysosomal fusion resulting in the accumulation of significantly larger vesicles with a higher [Ca^{2+}] [245]. TRPML1 was upregulated during nutrient starvation [246] and is important for the centripetal movement of lysosomes after autophagy induction [247]. In cardiomyocytes, TPCs seem essential for both basal and induced autophagy [248]. Moreover, TPCs can be regulated by the leucine-rich repeat kinase 2 (LRRK2). The latter is a multifunctional protein, of which various mutated species have been implicated in various diseases and Parkinson's disease in particular. In the cell, it participates in the control of various important processes including autophagy [249]. It now appears that LRRK2 stimulates NAADP-induced Ca^{2+} release from the lysosomes [241] (Fig. 7.3b). Furthermore, the aberrant lysosomal morphology observed in fibroblasts from patients harboring the LRRK2 G2019S mutation—the most prominent one in Parkinson's disease- can be rescued by TPC2 inhibition or knockout [250]. The local role of Ca^{2+} in this process is underpinned by the fact that BAPTA-AM, a fast intracellular Ca^{2+} buffer, but not EGTA-AM, a slow intracellular Ca^{2+} buffer, could also rescue the aberrant lysosomal morphology.

Finally, nutrient starvation induced TRPML1-mediated Ca^{2+} release out of the lysosomes, locally activating calcineurin [251]. Calcineurin subsequently dephosphorylated transcription factor EB (TFEB) leading to its nuclear translocation. Importantly, TFEB is a master regulator of many genes of the lysosomal/autophagic pathway [252], including the gene for TRPML1 itself [253, 254]. This demonstrates that TRPML can mediate its own upregulation through Ca^{2+}-dependent activation of TFEB. Moreover, this upregulation appears essential for adapting lysosomes to conditions of nutrient starvation [246].

These results indicate that the IP_3R, together with the lysosomal Ca^{2+} channels, participate in the occurrence of local Ca^{2+} signals in the microdomain between ER and lysosomes and can thus impact autophagy progression at various levels.

7.5 Conclusions

The IP_3R, by evoking intracellular Ca^{2+} signals with a specific spatio-temporal profile, is well known to regulate multiple cellular processes, ranging from fertilization to cell death via control of differentiation/proliferation, metabolism, contraction, secretion and many other processes. The IP_3R, of which three isoforms exist, is finely regulated by a variety of cytosolic factors including Ca^{2+} itself and ATP, as well as by a multitude of regulatory proteins, including cell death and survival proteins and kinases and phosphatases. The intracellular localization of the IP_3R at ER-mitochondrial and at ER-lysosomal contact sites is however also very important for its fundamental role in regulating cellular bioenergetics, autophagy and apoptosis. The existence of Ca^{2+} microdomains at these inter-organellar interfaces and the possibility to locally control [Ca^{2+}] in these microdomains, distinct from the bulk

cytosolic [Ca^{2+}], allows for a versatile role of Ca^{2+} signaling in a cell's decision to engage cell death, cell survival or cell adaptation processes in basal and stress-related conditions.

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